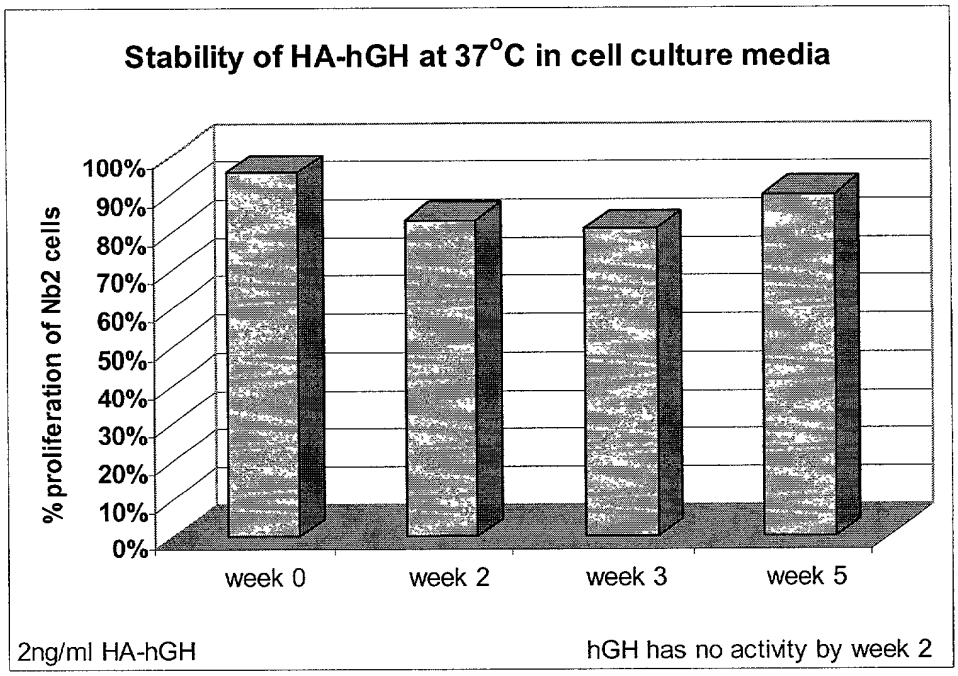

Stability of HA-hGH at 37°C in cell culture media
% proliferation of Nb2 cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
week 0
week 2
week 3
week 5
2ng/ml HA-hGH
hGH has no activity by week 2

Figure 1

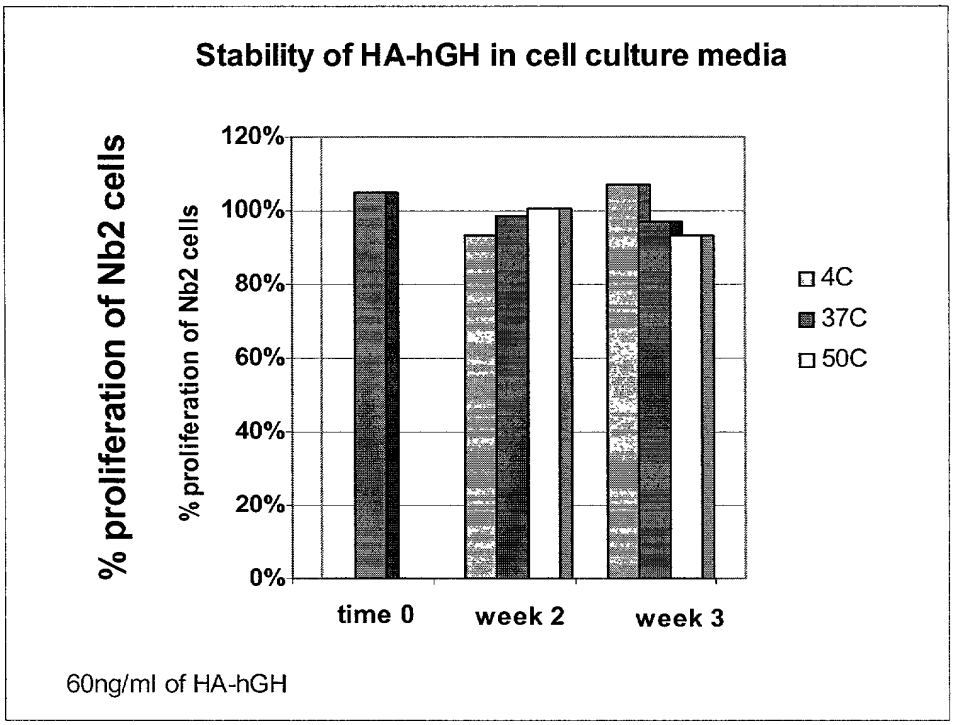

Stability of HA-hGH in cell culture media
% proliferation of Nb2 cells
% proliferation of Nb2 cells
120%
100%
80%
60%
40%
20%
0%
time 0
week 2
week 3
4C
37C
50C
60ng/ml of HA-hGH

Figure 2

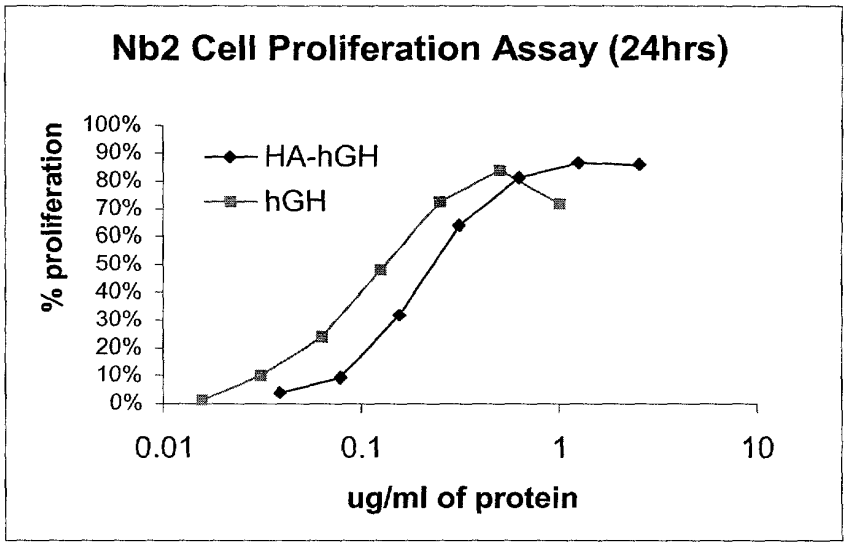


Figure 3A

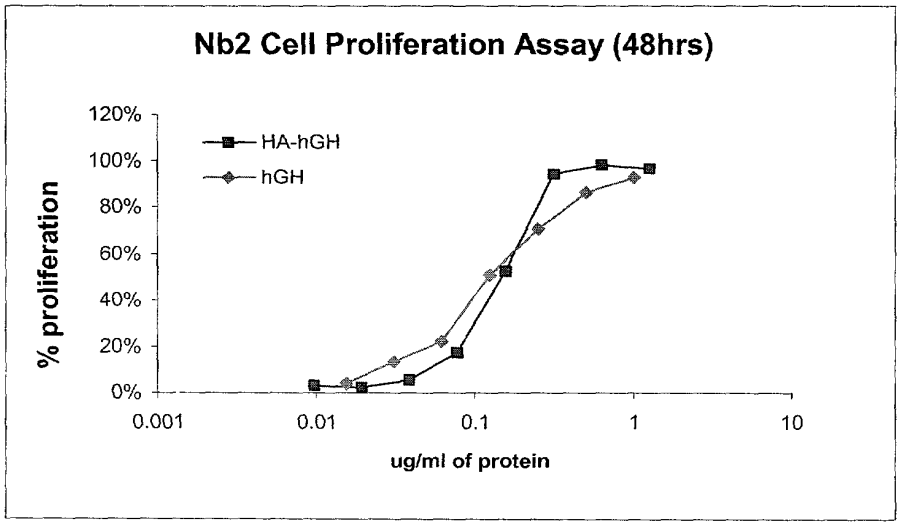


Figure 3B

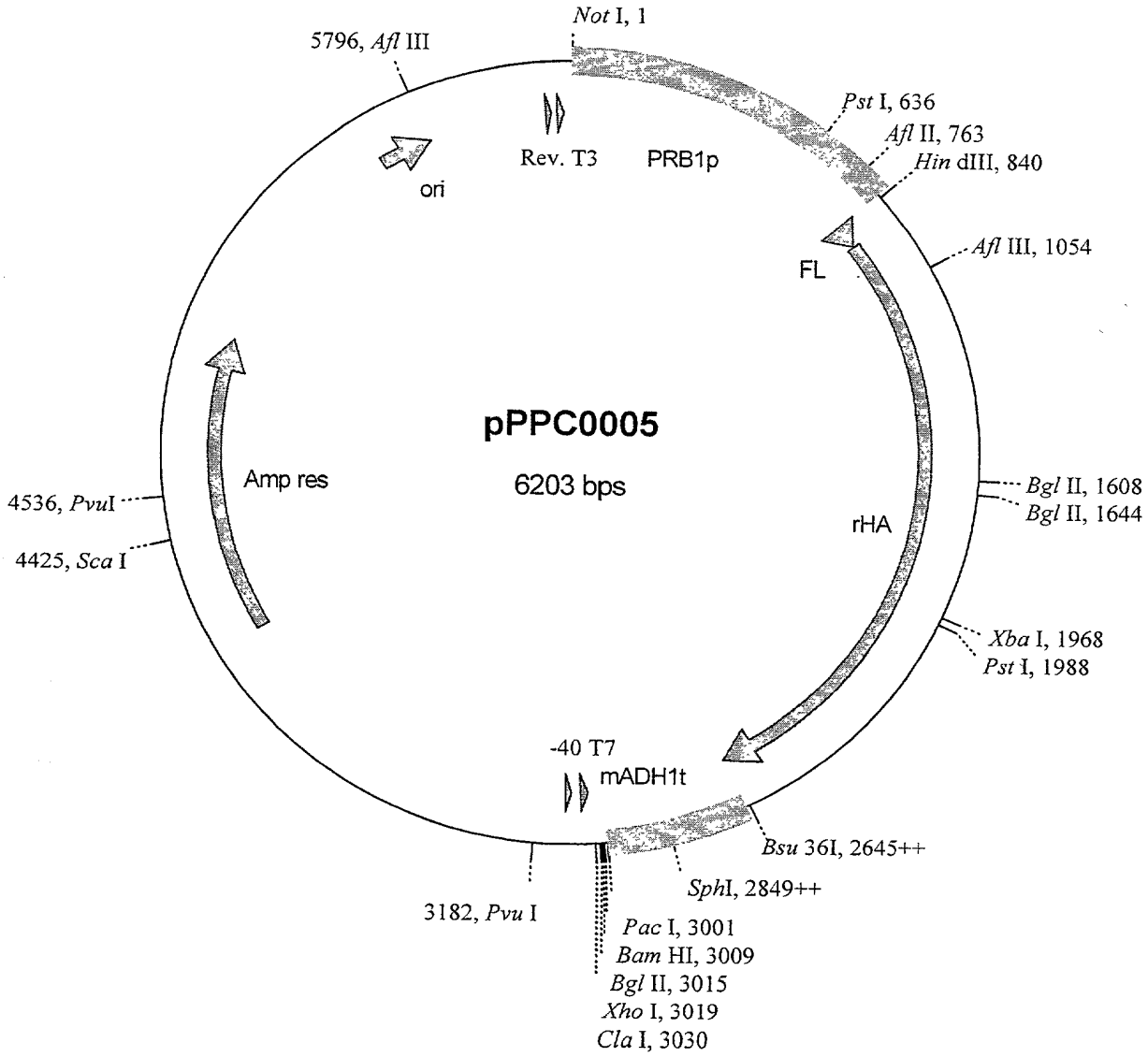

Not I, 1
5796, Afl III
Pst I, 636
Afl II, 763
Hin dIII, 840
Rev. T3
PRB1p
ori
Afl III, 1054
FL
pPPC0005
6203 bps
Amp res
4536, PvuI
4425, Sca I
Bgl II, 1608
Bgl II, 1644
rHA
Xba I, 1968
Pst I, 1988
-40 T7
mADH1t
Bsu 36I, 2645++
SphI, 2849++
3182, Pvu I
Pac I, 3001
Bam HI, 3009
Bgl II, 3015
Xho I, 3019
Cla I, 3030

Figure 4

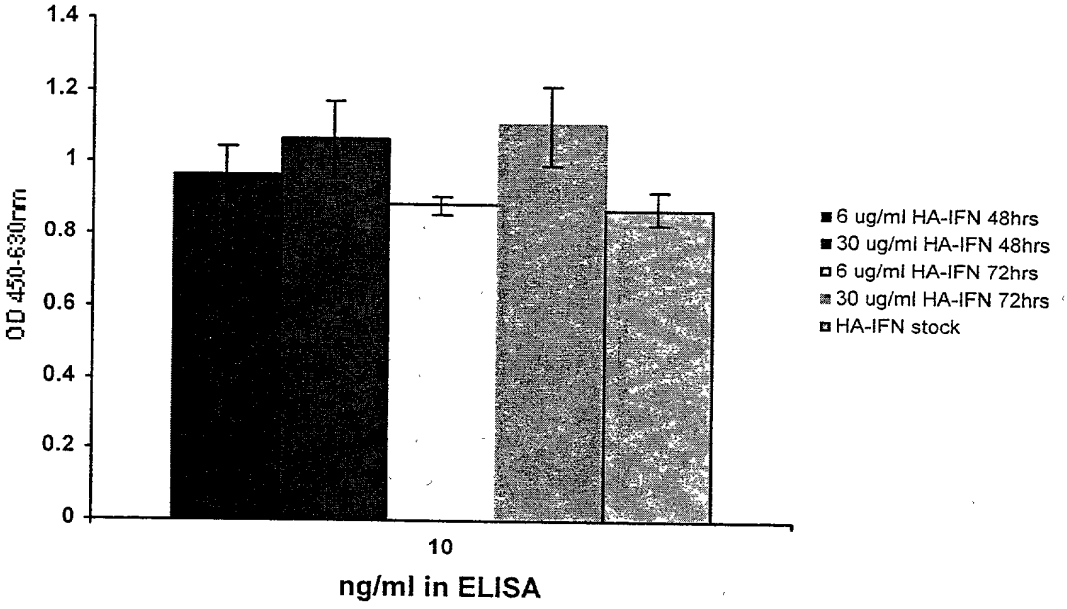

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450-630nm
10
ng/ml in ELISA
6 ug/ml HA-IFN 48hrs
30 ug/ml HA-IFN 48hrs
6 ug/ml HA-IFN 72hrs
30 ug/ml HA-IFN 72hrs
HA-IFN stock

Figure 5

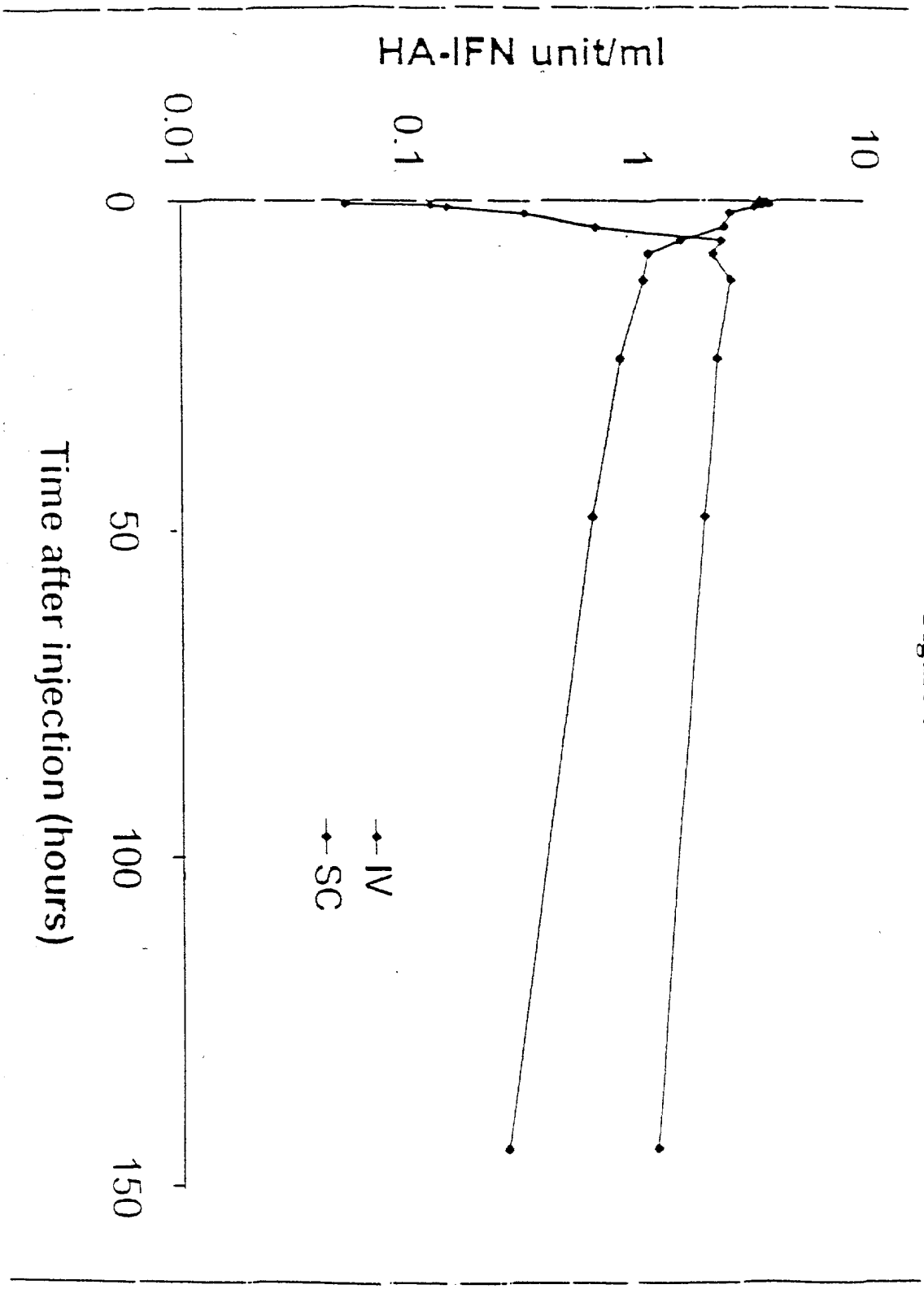

HA-IFN unit/ml
0.01
0.1
1
10
0
50
100
150
Time after injection (hours)
IV
SC

Figure 6

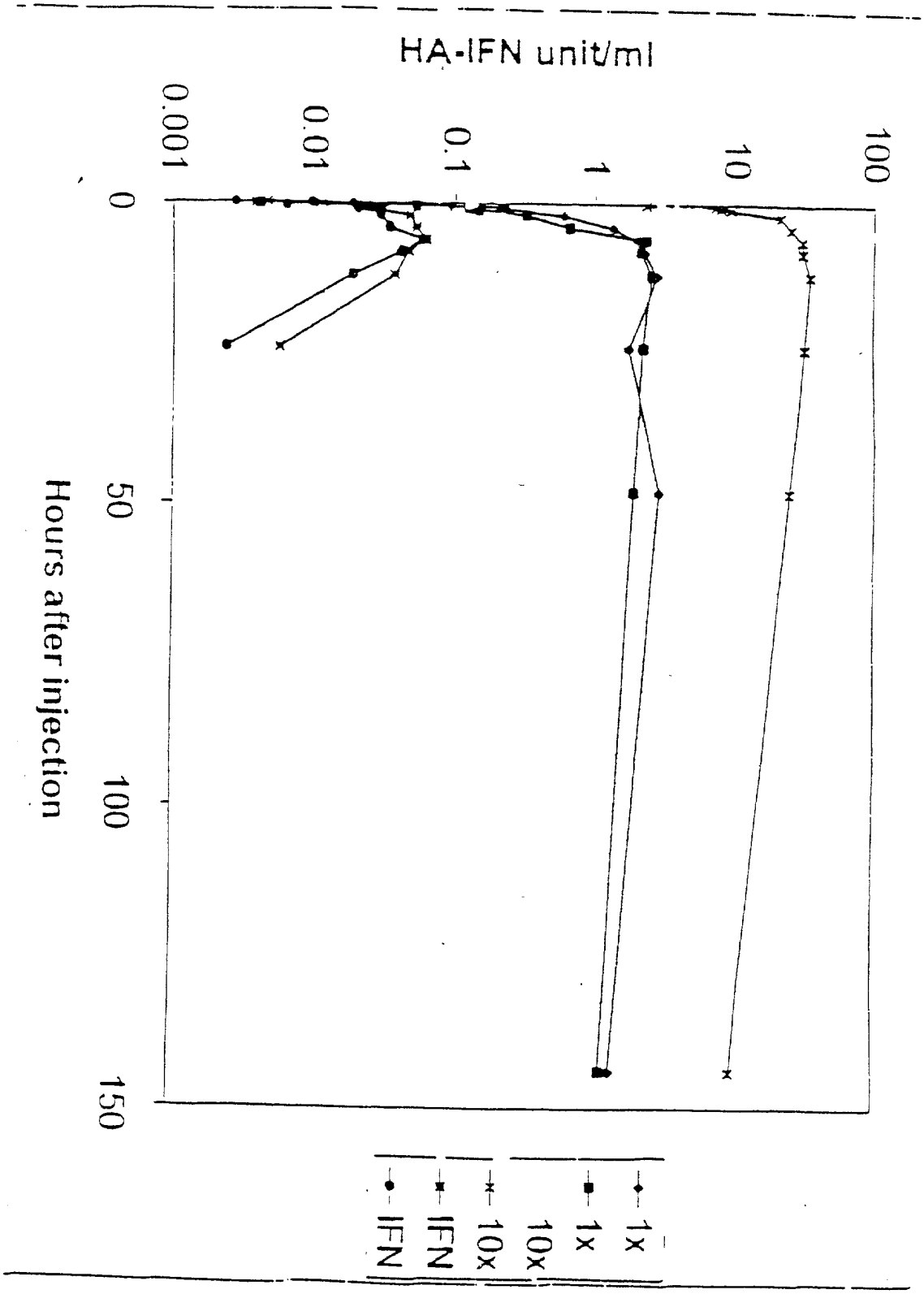

HA-IFN unit/ml
0.001
0.01
0.1
1
10
100
0
50
100
150
Hours after injection
1x
1x
10x
10x
IFN
IFN

Figure 7

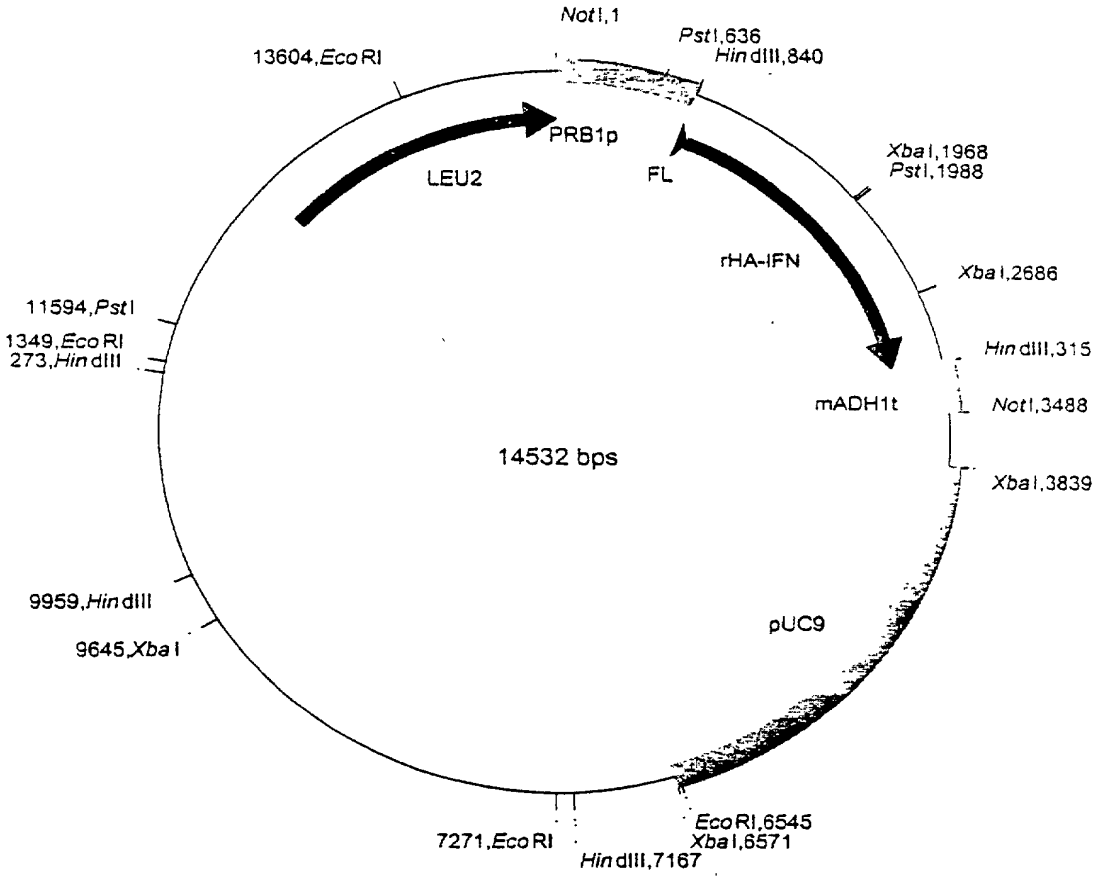


Figure 8. The HA-IFNα expression cassette in pSAC35. The expression cassette comprises
*PRB*1 promoter, from *S. cerevisiae.*
Fusion leader, first 19 amino acids of the HA leader followed by the last 6 amino acids of the MFα-1 leader.
HA-IFNα coding sequence with a double stop codon (TAATAA)
*ADH*1 terminator, from *S. cerevisiae.* Modified to remove all the coding sequence normaly present in the *Hin*d III/*Bam*HI fragment generally used.

Figure 8

Localisation of 'Loops' based on the HA Crystal Structure which could be used for Mutation/Insertion

```
1     DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
            HHHHH HHH     HHH HHHHHHHHHH       HHHHH HHHHHHHHHH

                I                            II              III
51    KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
      HHHHH             HHHHH HHHHH               HHHH H      HHHH

101   CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
      HHHH                 H HHHHHHHH     HHHHHHHHH HHHHH

                                   IV
151   APELLFFAKR YKAAFTECCO AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHEHHHHHH HHHHHHHHHH

                                                          V
201   ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
      HHHHH   HH HHHHHHHHHH HH      HHH HHHHHHHHHH HHHHHH  HH

                            VI                VII
251   LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
      HHHHHHHHHH HHHHH           HHHHH     HHHHHHH H

301   DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
            HHHH      HHHHHH    HHHHHHH HHHHHH       HHHHHHHH

                    VIII
351   KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
      HHHHHHHHHH       HH    H   HHHHH HHHHHHHHHH HHHHHHH

                                                      IX
401   YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
      HHHHHHHHHH HHHH     H HHHHHHHHHH    HHH       HHHHHHHH

                          X                   XI
451   DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
      HHHHHHHHHH HHHHH      HHHHHHHHH     HHHHHHHH

501   EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
                  HHH   HHH HHHHMMEHHH HHH          HHHHHHHH

                   XII
551   FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
      HHHHHHHH         HHHH HHHHHHHHHH HH
```

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 9

Examples of Modifications to Loop IV

a. Randomisation of Loop IV.

```
                              IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH

                              IV
151   APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

$(X)_n$

↓

IV

```
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH        HHHHH HHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

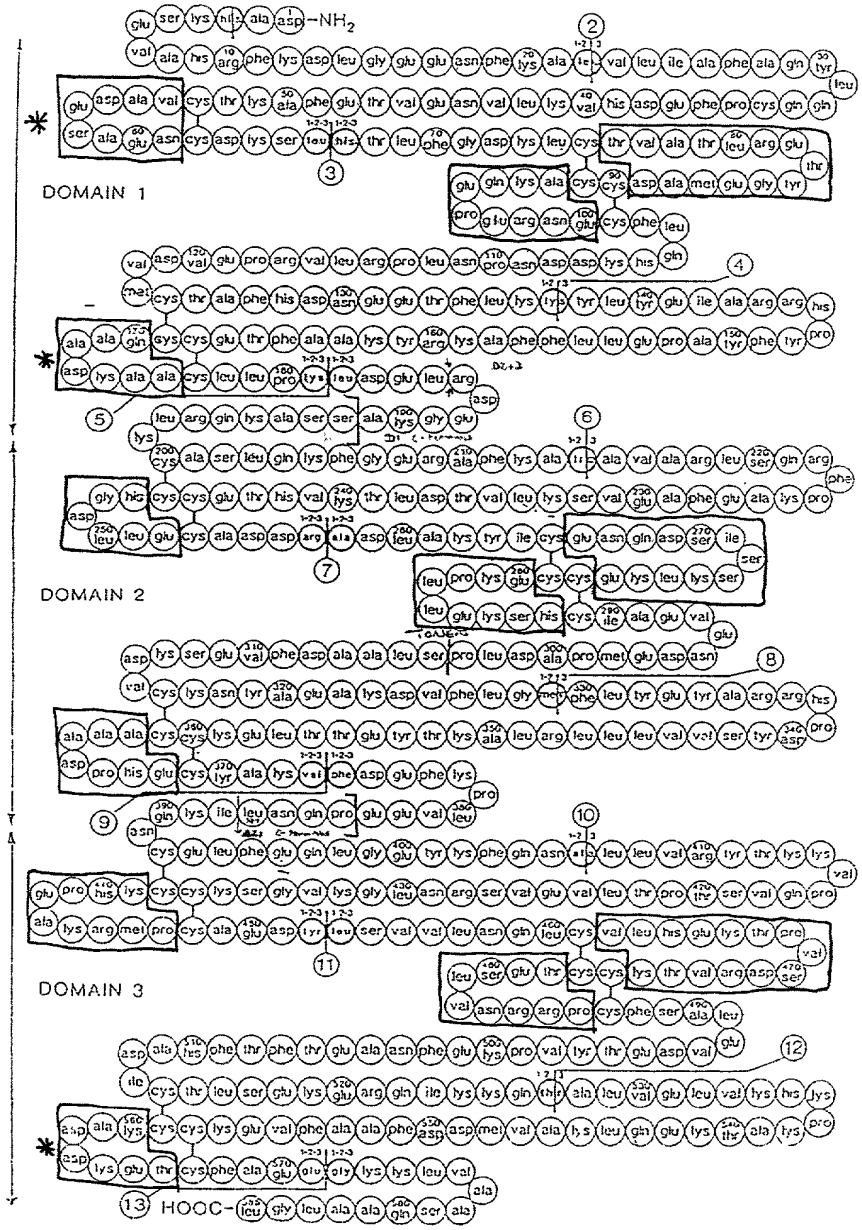
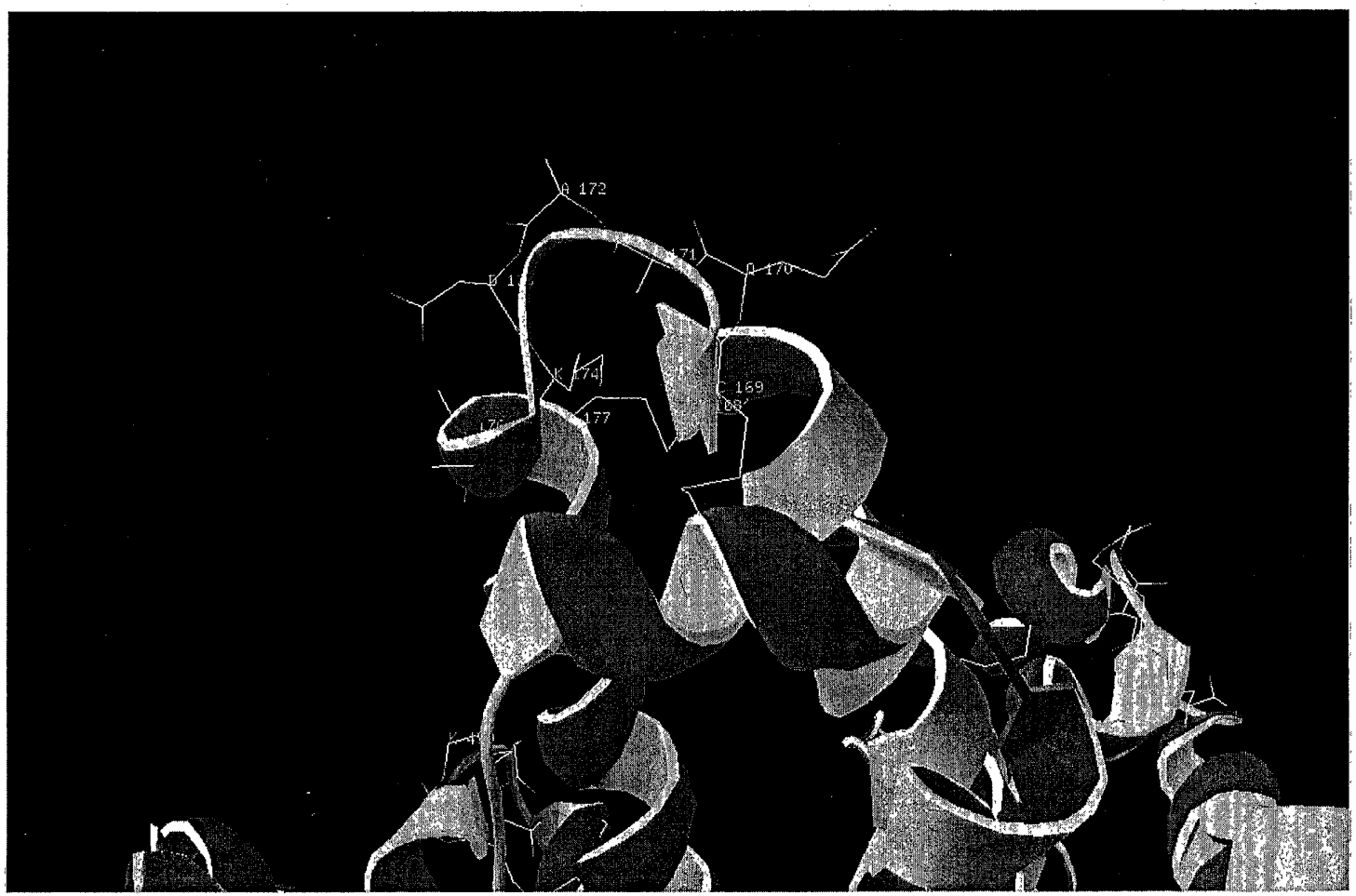


Figure 11



Disulfide bonds shown in yellow

Figure 12: Loop IV Gln170-Ala176

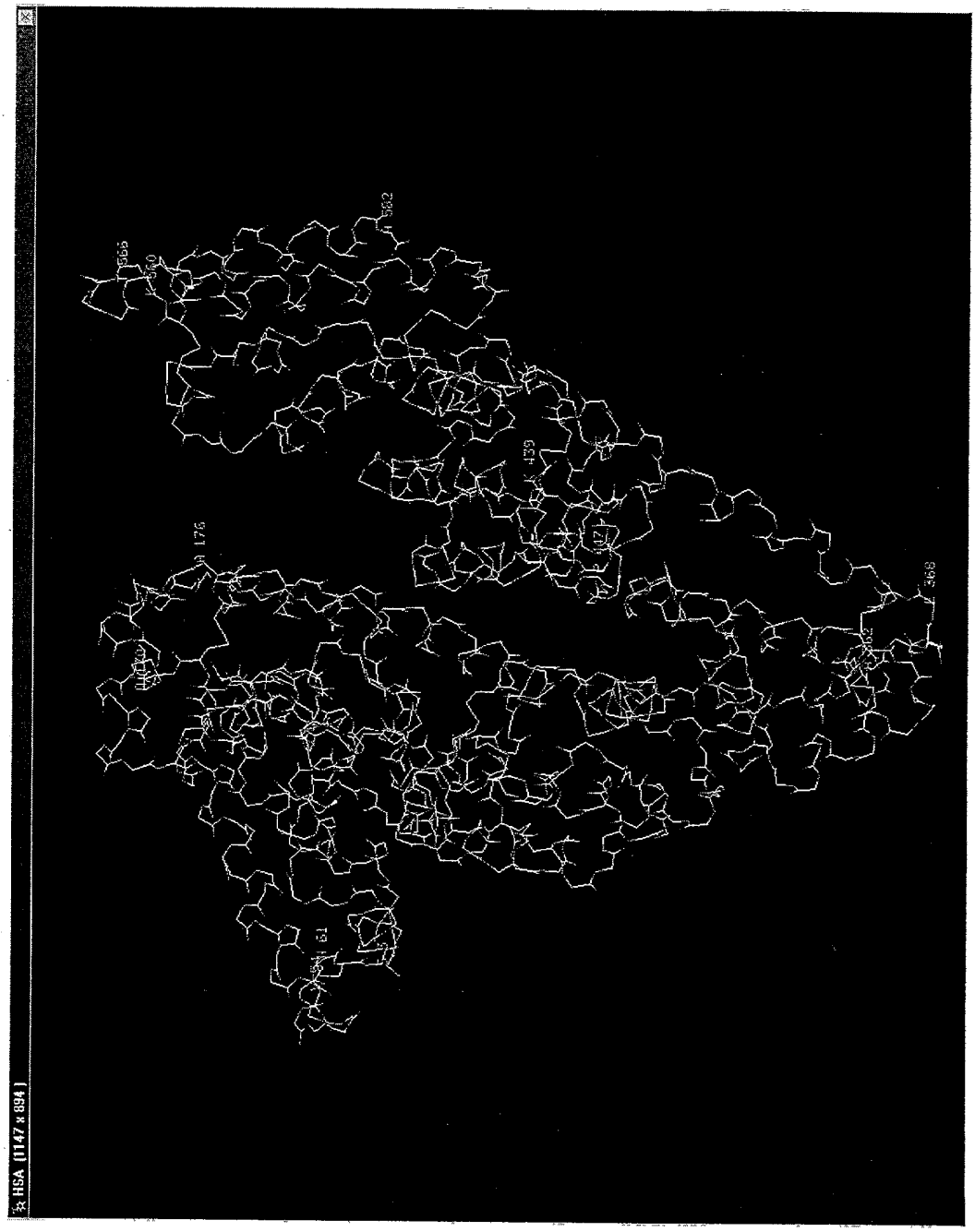


Figure 13: Tertiary Structure of HA

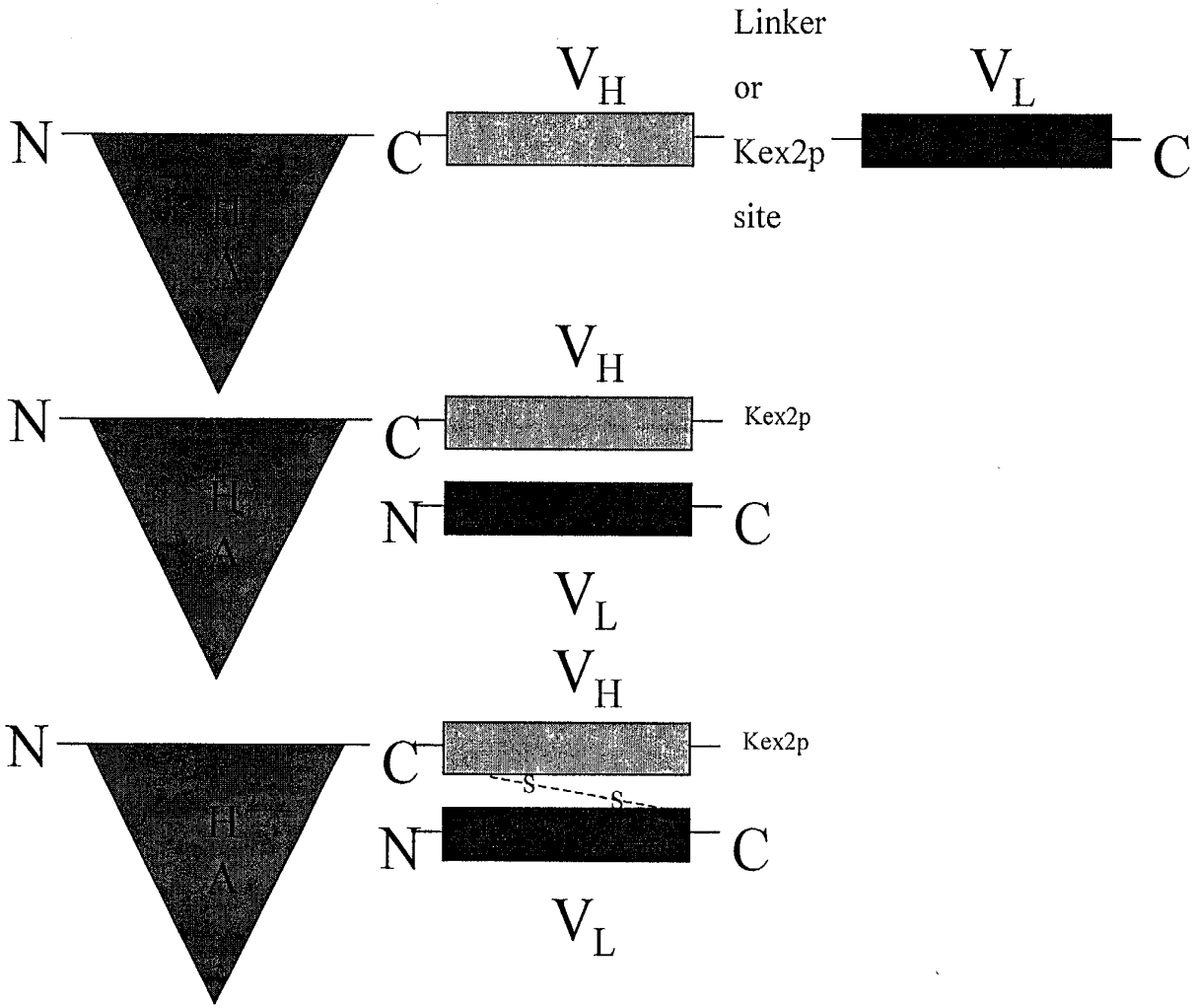


Figure 14: Schematic Diagram of Possible ScFv Fusions (Example is of a C-terminal fusion to HA)

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K   20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V   40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E   60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L   80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

Figure 15A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 15B

```
 961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
```

Figure 15C

```
1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L   V   N   R   R   P   C   F   S   A   L   E   V   D   E   T   Y   V   P   K   500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                                   585
```

Figure 15D